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The HPRT Short-Term Assay in Monitoring Individuals Exposed to Genotoxic Agents

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This paper reviews several monitoring studies where the short-term HPRT assay has been applied. The original method uses autoradiography to detect ^{3}H-thymidine incorporation in variant cells that have undergone DNA synthesis; the bromodeoxyuridine modification employs this thymidine analog and fluorescence plus Giemsa staining. The studies discussed here were accomplished with either of these methods. methods. Exposures analyzed include radiation and chemotherapy as medical treatments and accidental exposures to radiation; these studies have been useful in the validation of the assay because radiation and anticancer drugs are well-known mutagens. Other potential mutagens such as environmental arsenic and a parasitic infection and praziquantel, used for its treatment, have also been monitored for *hprt* locus mutation. An overview of the results obtained with different agents and routes of exposure is presented here as well as some methodological aspects for the optimization of the assay for monitoring studies.

Introduction

The HPRT T-lymphocytes assay is the most widely used gene mutation assay for human monitoring studies (*1*). The long-term cloning assay measures the frequency of thioguanine-resistant (TG^r) T-lymphocytes and simultaneously allows the recovery and further analysis of individual clones to measure the mutation frequency (MF). The exact nature of the *hprt* mutation can be defined by Southern blotting and DNA sequencing (*1*).

The short-term assay can be done either by autoradiography to detect tritiated thymidine (^{3}H-Tdr) incorporation in cells that have undergone DNA synthesis (*2*) or by the bromodeoxyuridine (BrdUrd) modification, which uses this thymidine analog and fluorescence plus Giemsa (FPG) staining (*3*). These short-term assays have been proposed as useful for population monitoring because they are relatively inexpensive, simple, and available to any cytogenetic laboratory.

The HPRT Short-Term Assay

Detailed methods have been described elsewhere (*2,3*). Briefly, peripheral blood lymphocytes separated by Ficoll-Hypaque centrifugation are cryopreserved at −70°C in RPMI 1640 medium with 10% autologous plasma and 7.5% dimethyl sulfoxide at a cellular density of 10^7 cell/mL. The cultures for thioguanine (TG) selection are set up after thawing the cells at a density of 5×10^6 cells/mL in RPMI-1640 with 20% autologous plasma, stimulated with phytohemagglutinin and incubated at 37°C for 24 hr. Twenty microliters of ^{3}H-Tdr (6.7 Ci/mmole) or 200 μL of BrdUrd (4×10^{-4} M final concentration) are added for the last 16 hr of culture. Incubation is ended with 0.1 M citric acid, and cultures are fixed with methanol:acetic acid (7:1.5).

Slides for microscopic evaluation are prepared and processed for autoradiography or are stained by the FPG method. The labeling index (LI_c) of control preparations is calculated as follows: LI_c = no. of labeled nuclei/5000 evaluated nuclei. In the TG culture preparations, LI_t = no. of labeled nuclei (variants)/total number of nuclei recovered from TG cultures. The variant frequency (VF) index is then calculated as LI_t/LI_c.

Quantitative Measurements of Variant Frequency in Human Somatic Cells

The backgrounds for normal adults determined by the autoradiographic or the BrdUrd assay, using cryopreserved cells, are listed in Table 1. These studies, whether using ^{3}H-Tdr or BrdUrd, show similar mean VF values (5.9×10^{-6} and 4.82×10^{-6}, respectively). These values are comparable to the mean MF found in studies where the cloning assay was used (5.25×10^{-6}). Values reported by several authors (*1,3,5,8*) show differences, and a distribution of variant frequencies has been found. Considering all

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Table 1. Variant frequencies (^{3}H-Tdr and BrdUrd methods): controls.

Exposure	Type of assay	No. of samples	Mean frequency ($\times 10^{-6}$) + SD	Range ($\times 10^{-6}$)	Reference
Control	^{3}H-Tdr	18	7.12 + 7.16	0.50–22.3	(3)
Control	^{3}H-Tdr	82	8.70 + 6.10	1.00–28.9	(1)
Control	^{3}H-Tdr	8	1.92 + 0.85	0.68– 3.24	(5)
		Total average = 5.9			
Control	BrdUrd	2	4.31 + 1.68		(3)
Control	BrdUrd	5	6.35 + 1.53	4.20– 8.40	(8)
Control	BrdUrd	15	3.15 + 4.28	0.52–14.13	This paper
		Total average = 4.82			
	Cloning	252	5.25		(1)[a]

[a]Calculated from Table 1 from Albertini et al. (1).
the studies together, the range of values is from 0.0 to 28.9 $\times 10^{-6}$. In each study, different means have been found, thus there is also a range of mean values from 1.92 $\times 10^{-6}$ to 8.7 $\times 10^{-6}$.

Significant increases of VFs have been reported in cancer patients treated with chemotherapy or radiotherapy (4–6) or in individuals exposed in radiation accidents (Table 2) (7,8). These studies have been useful in validating the assay. With respect to radiation, as with other end points, data obtained on the VF values show a clear-cut effect in the case of accidental exposures (7,8). Patients receiving localized and fractionated radiotherapy (4) also showed clear increases of VF, but smaller increases than in the case of accidental exposure. The range of data found in this study is 0.0–27.8 $\times 10^{-6}$. The highest value corresponds to those in the range found for healthy controls in other studies (Table 1), but since this was a longitudinal study started before treatment and followed every month, differences in VF could clearly be observed because each individual served as his or her own control.

In a study on patients receiving chemotherapy and/or radiotherapy, a clear increase in the range of VFs was found: from 0 to 135 $\times 10^{-6}$ in comparison to controls, which showed a range of 0–16.5 $\times 10^{-6}$. Some of the treated patients had a VF value greater than the highest value of controls, as expected since these drugs and radiation are well-known mutagens and their effect should be clearly detected; nevertheless, a high percentage of the individuals exposed showed VF values in the control range (more than 50%).

In the case of exposure to a single chemical, the well-known cytostatic drug cyclophosphamide has been studied in a group of workers involved in its production and in patients under treatment for multiple sclerosis (5,9). In the first case, a clear increase in VF was found in the exposed workers (data are not included due to noncomparable VF values because cells were not cryopreserved). In the study of multiple sclerosis patients, five showed a clear increase in VF after 14 days of treatment, and only one did not show an increase (probably due to trimethoprim treatment).

Chronic exposure to arsenic in drinking water did not show an effect on the variation frequency of *hprt* locus (10). In general, VF in the exposed group were higher than those found in the low-exposure group (mean ± SD: 5.03 $\times 10^{-6} \pm 2.9$, 2.48 $\times 10^{-6} \pm 2.2$, respectively), but all the values are in the range found for the control, unexposed group (from 0.52 $\times 10^{-6}$ to 14.13 $\times 10^{-6}$) (Fig. 1, Table 1 and 2). Statistically, no significant difference was found among any of these groups.

Mean variant frequencies among neurocysticercotic patients, exposed to several mutagenic treatments (anticonvulsants, computerized axial tomography, anesthetics, antibiotics, and anti-inflammatory drugs) show signifi-

Table 2. Variant frequencies (^{3}H-Tdr and BrdUrd methods): exposed individuals.

Exposure	Type of assay	No. of samples	Mean frequency ($\times 10^{-6}$) ± SD	Range	Reference
Radiation accident					
Cobalt-60	3H-Tdr	2	122.0 ± 5.9	18.6 –126.2	(11)
Cesium-137	BrdUrd	4	194.0 ± 147.0	36.0 –130.0	(8)
Cancer patients					
Pretreatment	3H-Tdr	10	3.0 ± 1.7	0.0 – 6.1	(4)
Radiotherapy	3H-Tdr	9	12.6 ± 8.7	2.5 – 23.8	(4)
Chemotherapy plus radiotherapy	3H-Tdr	35	24.00[a]	0.0 –135.0	(7)
Multiple sclerosis (MS)					
Pretreatment	3H-Tdr	6	4.07 ± 3.1	0.83– 9.9	(5)
MS + cyclophosphamide	3H-Tdr	4	32.53 ± 13.97	11.6 – 40.3	(5)
Low As	BrdUrd	7	2.48 ± 2.19	0.68– 6.36	(11)
High As	BrdUrd	7	5.03 ± 2.99	1.66– 9.65	(11)
Neurological patients	BrdUrd	6	9.49 ± 6.25	3.33– 20.79	This paper
Neurocysticeroctic (NC) patients[b]	BrdUrd	22	14.48 ± 24.89	1.46–101.01	This paper
NC + praziquantel	BrdUrd	12	17.55 ± 27.10	3.03–102.51	This paper

[a]Deviation could not be determined from the original paper.
[b]These patients were exposed to several mutagenic agents before praziquantel treatment.

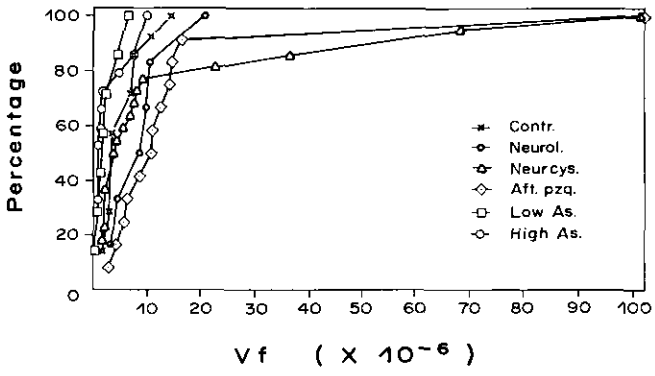

FIGURE 1. Cumulative variant frequencies (VF) found in individuals exposed to different agents. Controls are found among the lowest values in the scale; exposed persons distribute widely.

cantly increased VF values in relation to healthy controls: 14.48×10^{-6} and 3.15×10^{-6}, respectively (Fig. 1, Tables 1 and 2). These patients did not statistically differ from an appropriate control group, which consisted of neurological patients exposed to the same mutagenic treatments, but who were not infected.

Seven of the 22 patients could be studied before and after praziquantel treatment: 4 had a lower VF after treatment, 2 did not show any significant difference, and 1 increased. Mean value before treatment was 28.78×10^{-6} and after treatment was 22.4×10^{-6}; these values are different from controls, but there is no significant difference between them (Fig. 2).

Some Methodological Aspects of Monitoring Studies

An appropriate study design is fundamental. For example, in the case of the radiotherapy study of Ammenheuser et al. (*4*), effects could be observed because sampling was performed before, during, and after treatment, allowing the observation of a dose-related response due to the cumulative effect characteristic of radiation. When studying chemicals, this type of design is difficult to accomplish; in the best of the cases, patients are sampled only before and after treatment, or a control group is compared to an exposed group. When these protocols are used, overlapping ranges are usually found, so that most of the exposed individuals cannot be distinguished from normals.

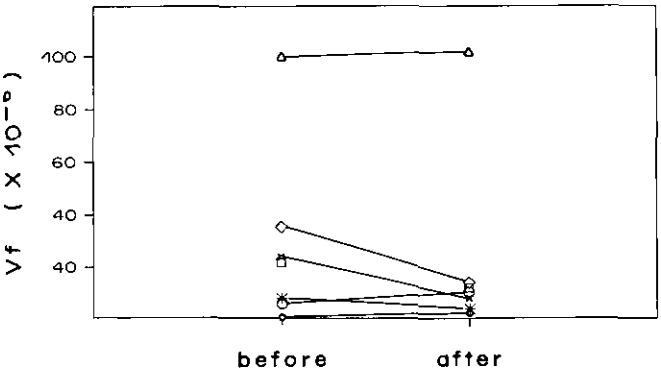

FIGURE 2. Variant frequency values in seven neurocysticercotic patients before and after praziquantel treatment.

Sampling time is another factor discussed by Ammenheuser et al. (*4,5*) when studying individuals exposed to medical treatments. At least 15 days would be necessary for the mutations produced by exposure to be manifested in cells, and once treatments ended, there would be a period during which mutation frequency increases could be detected before the mutant cells are eliminated from the body by homeostatic mechanisms, as suggested from their studies (*4,5*).

But generalizations cannot be made because there are exposures that apparently produce more persistent damage as was observed with radiation accidents where samples were taken 17 months (*7*) and 24 months (*8*) after the accident and still showed high VF values.

Interpretation of Results

Interpretation of effects is not an easy task due to the variations in control values from healthy subjects, as already stated. Controls generally are grouped around the smallest values and when there is an exposure, these values become less frequent. It has been proposed that human population monitoring for mutagenicity is concerned with group means, and in identifying individual values that are above the range for normals (*1*). Nonetheless, variability raises several questions about the range to be considered normal, and if means could be used to compare different groups, and which values should be considered positive due to exposure. Should a small shift in the distribution of values of exposed people be interpreted as a risk for health, or should we expect no overlapping to consider positive an effect? It should be borne in mind that it has not been established which amount and what kind of damage is hazardous for health.

Although there is an overlap of VF ranges between controls and exposed groups, the way they distribute is different, i.e., more elevated VFs are found in more individuals so that distributions are shifted toward higher mean values (Fig. 1) when there is an exposure. It is interesting to point out that the "outliers" with exceptionally high VFs may represent more susceptible individuals. This susceptibility could be due to pharmacogenetic differences (*11,12*) and should be investigated further. In trying to identify risk groups, we often forget that individuals are the ones who suffer cancer and reproductive damage; the obvious question is if outliers are the individuals prone to genetic health effects.

The need for studies of larger groups should be stressed, but this can only be achieved by speeding up microscopic analysis, which is time consuming. Computer image analysis or cytofluorometry are viable solutions, and in this respect it has recently been applied an immunohistochemical antibromodeoxyuridine staining for determination of 6-TG^r T-lymphocytes, which has the potential for automation (*13*).

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